



Recommended Minimum Reporting Information For Publication of Eco-transcriptomics Studies

In the last decade, there have been prolific efforts to develop, enhance, and implement new approach methods (NAMs), motivated by the reduction, refinement, and replacement of vertebrate animal testing. Transcriptomics, among the other “-omics” disciplines, has become more accessible as costs continue to decrease and the computational power required to process and analyze data increases; however, the application of transcriptomics into regulatory frameworks and risk assessment has not paralleled research efforts. Prior to formal analysis, inconsistencies in experimental methods and the reporting of appropriate metadata drive ambiguity in results by limiting reproducibility and comparability. As the rate of new transcriptomics studies continues to increase, it is imperative that transparency in reporting also improves. Doing so will facilitate the use of these data sources in risk assessment.

Transparent Reporting of Experimental Variables Increases Confidence, Reuse, and Impact of Results

SETAC has consistently advocated for both open data – data that are accessible and reusable for reanalysis or metaanalysis without additional context – and open science in general. While data from transcriptomics studies closely mirrors previous recommendations for reporting information on environmental toxicity studies, methodological reporting of crucial steps such as quantification, library preparation, and sequencing parameters is often

limited. Failure to report such methodological information introduces unknown variables. In consequence, data are limited in their comparability, whether it is across studies, laboratories, or species. Additionally, reporting bioinformatic workflow is critical, though not the focus of the current communication.

Concerted Change Requires Harmonized Efforts

Inconsistency in scientific reporting is not a novel subject. Multiple recommendations for transcriptomic reporting have been published, such as MINSEQE or the OECD's Omics Reporting Framework. However, many researchers rely on sequencing partners (e.g., academic institutions or private sector) and may not receive applicable metadata or know to ask for it. While many journals have adopted the requirement to provide a data availability statement or data transparency policy, these policies do not completely prevent insufficient reporting. Concerted effort among researchers, sequencing partners, and journals is necessary to set a precedent. While other limiting factors exist in addition to concerns mentioned here – such as a lack of standardized approaches to data processing (e.g., deriving of a concentration at which a concerted molecular change occurs, such as a transcriptomic point of departure or biological pathway altering concentration) and standardized minimum acceptability criteria (e.g., a minimum number of replicates, positive and negative controls, etc.) – the harmonization of data reporting will enhance confidence in published data, advance NAMs, increase cross-comparability, and improve animal welfare.

Recommended Minimum Reporting Information of Eco-Transcriptomics Studies

To facilitate transparent data reporting, we have suggested a list of minimum information to be reported in studies measuring transcriptomic endpoints based on a subset of elements from the Organisation for Economic Co-operation and Development Omics Reporting Framework (OORF).

The suggested list is not all inclusive and depends on the type of study being reported, yet it acts as a starting point to improve reporting. This list can be easily adapted to in vitro experiments by substituting animal husbandry information for cell culture conditions.

Test Subject

Test organism description:

- » Indicate scientific name, strain, and source if transgenic.
- » Provide a clear description of organism origin for the experiment. Specifically, if the organism was obtained from in house culture, a commercial facility, wild caught, etc.
- » State the life stage used for the experiment (i.e., embryonic, larval, juvenile, adult), and report the age at experiment start, if known. For experiments using embryos or larvae, the age of the brood stock should also be supplied.

- » Make it clear the experiment is using males, females, both sexes at a fixed proportion, both at an unknown proportion, or any known combination.

Animal husbandry before and during experiment:

- » Describe the acclimation period prior to exposure conditions, if applicable.
- » Report measured parameters both before and during exposure (e.g., light:dark cycle temperature, pH, etc.).
- » Explain if organisms were fed before and during exposure. Describe the feeding regimen including food that was used.

Experimental Design

Replicates (biological and technical):

- » For each method, report the number of biological replicates used in each treatment and if there was any sample pooling. Be specific about where pooling occurred in the process. Sometimes due to tissue limitations, this may mean different biological replicates for the experiment, versus the sequencing. This needs to be clearly reported.
- » Report the number and setup of replicates for control group(s). Clearly state whether a water control, a solvent control, or both are used.

Stressor:

- » State the chemical name, including the chemical abstracts services (CAS) number and purity.

- » If a chemical is dissolved in a solvent, then report the solvent CAS and purity, as well as the concentration at which it is prepared (stock concentration).
- » Report the exposure schedule (e.g., duration, frequency, and time of day).
- » Report the route of exposure (e.g., dietary, injection, waterborne, etc.).
- » Provide some information on verifying test concentrations, otherwise include lack of verification as a limitation of the study.
- » Report all nominal and measured (if applicable) concentrations used within the experiment clearly in the methods section.

Transcriptomic Methodology

RNA extraction:

- » Total RNA quantity and purity should be reported. This can include output from NanoDrop, Qubit, Bioanalyzer, TapeStation results, etc. Purity and quality

values for either the full experiment or all samples should be reported using methodology specific output (e.g., RNA integrity number values, A260/280, A260/230), whereas quantity should be reported as

ng/ μ L, μ g/ μ L, or total ng/ μ g (i.e., RNA quantity in the total volume eluted during nucleic acid extraction).

Library Preparation:

- » Any Genomic DNA removal, rRNA removal (ribodepletion), or mRNA enrichment must be reported. Often these steps are in the RNA extraction or library preparation kits; authors should clearly state which kit was used, which optional steps were applied, and which modifications were made.
- » Describe the sequencing library type and kit.
- » Report whether preparation was performed with total RNA or mRNA, and the amount used for library preparation.
- » Provide the number of cycles ran during PCR amplification.

fication.

- » State if equivalent concentrations of RNA/mRNA/cDNA for each treatment are being used during all steps of library preparation or if this is being assumed but not tested. Report if samples are initially standardized or if concentrations are verified throughout library preparation steps.
- » Provide the concentration and volume of the final loaded library.

Sequencing:

- » Report the sequencing platform name and whether methods were single or paired-end reads.
- » Provide sequencing depth.

Data Availability

Reproducibility:

- » Deposit data in a central accessible repository (i.e., National Center for Biotechnology information or NCBI), and provide corresponding accession number in addition to pertinent metadata (i.e., file name, replicate, treatment, etc.) to allow for reanalysis if desired. If not, provide text explaining why data is not available.
- » Include a copy of the completed table (link below) as supplemental information when submitting for publication.

Resources

A supplemental table for reporting the information listed above can be found at <https://doi.org/10.6084/m9.figshare.28807160>.

The OECD Omics Reporting Framework can be found at https://www.oecd.org/en/publications/oecd-omics-reporting-framework-oorf-guidance-on-reporting-elements-for-the-regulatory-use-of-omics-data-from-laboratory-based-toxicology-studies_6bb2e6ce-en.html.

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